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Published in:
Applied Animal Behaviour Science

DOI:
10.1016/S0168-1591(01)00200-3

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2002

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
van Loo, P. L. P., Kruitwagen, C. L. J. J., Koolhaas, J. M., van de Weerd, H. A., van Zutphen, L. F. M., & Baumans, V. (2002). Influence of cage enrichment on aggressive behaviour and physiological parameters in male mice. *Applied Animal Behaviour Science*, *76*(1), 65-81. https://doi.org/10.1016/S0168-1591(01)00200-3

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Applied Animal Behaviour Science 76 (2002) 65–81

APPLIED ANIMAL BEHAVIOUR SCIENCE

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Influence of cage enrichment on aggressive behaviour and physiological parameters in male mice

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Accepted 11 October 2001

Abstract

From welfare perspective group housing of mice is preferred over individual housing. Group housing of male laboratory mice, however, often leads to problems due to excessive aggressive behaviour. In our search for management and housing modifications to decrease aggression in group-housed male laboratory mice, we have tested the effect of two types of environmental enrichment—nesting material and shelter—on aggressive behaviour after cage cleaning and after a 1 h isolation period. Severity of wounds, urinary corticosterone levels, body weight, food and water intake and several post-mortem parameters were also monitored.

The results indicated that type of enrichment strongly affected both aggressive behaviour and physiological parameters. Overall, nesting material reduced aggressive behaviour, while a shelter increased aggressive behaviour compared to control housing. This effect was also reflected in the number of wounds counted. Furthermore, during shelter housing mice gained less body weight, drank less and showed higher corticosterone levels, while in housing conditions with nesting material, mice ate less. We conclude that providing male mice with nesting material reduces aggression between male mice, and may, thus, be promoted as being beneficial to their physical health and psychological well-being. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Aggression; Environmental enrichment; Male mice; Stress; Welfare

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PII: S0168-1591(01)00200-3

1. Introduction

The use of environmental enrichment to improve the well-being of laboratory animals is promoted widely and is incorporated in European legislation (Council of Europe, 1997; Rodent Refinement Working Party, 1998; Kornerup-Hansen, 1999). The general aim of its use is to enhance species specific behaviour, promote physical health as far as possible and to decrease abnormal behaviour while keeping a focus on scientific, economic and ergonomic demands (Newberry, 1995; Dean, 1999; Baumans, 2000). A wide range of experiments concerning laboratory, farm and zoo animals has proven these benefits of environmental enrichment (Prior and Sachser, 1994/1995; Van de Weerd et al., 1997a,b, 1998; Würbel et al., 1998). There are, however, still objections against using environmental enrichment for laboratory animals.

Firstly, there is concern that the use of environmental enrichment of any kind may be a threat to the existing standardised control conditions as enriched housing conditions may influence both the absolute outcome and the variability of experimental results. Changes in the absolute outcome of results may invalidate historical data (Dean, 1999) and changes in variability in results may in some cases lead to an increase in the number of animals needed for research. In light of the general goal towards replacement, reduction and refinement with regard to the use of laboratory animals, the R of refinement would then counteract the R of reduction (Russell and Burch, 1959). Interest and research in this area is growing (Eskola et al., 1999; Van de Weerd et al., 2001). Secondly, controversy exists as to whether environmental enrichment induces an increase or decrease in aggressive behaviour between laboratory animals that are group housed. When male mice are housed socially under laboratory conditions, a certain level of aggression between male mice seems inevitable, as the situation is far from natural. In general, these males will form, mainly despotic, dominance relationships (Poole and Morgan, 1973; Mondragón et al., 1987). In many cases, depending on strain and age, the hierarchy in these groups is stable, while in other cases, aggression may reach levels at which individuals are wounded badly (Van Oortmerssen, 1971; Bisazza, 1981; Brain and Parmigiani, 1990). Several authors have indicated that environmental enrichment leads to an increase in aggression when male laboratory mice are housed together, and conclude that the enrichment may actually reduce their well-being in this respect (McGregor and Ayling, 1990; Haemisch and Gärtner, 1994; Haemisch et al., 1994). Others have found that cage enrichment or environmental complexity does not alter, or decreases the amount of aggression between male mice (Vestal and Schnell, 1986; Chamove, 1989; Ward and DeMille, 1991; Armstrong et al., 1998; Ambrose and Morton, 2000). In the field of farm animal welfare too, environmental enrichment has been used to reduce aggression in laying hens and growing pigs (Gvaryahu et al., 1994; O'Connell and Beattie, 1999). The existing controversy in the literature covering the effect of enrichment on aggression in male mice may be a result of the variety in experimental set-ups. In the studies mentioned, male mice were housed in group-sizes varying from 2 to 10. In some cases, the mice were acquainted, while in others they were not, and age of grouping or testing differed from weaning (3 weeks) to 18 weeks. Furthermore, measures of aggression differed between studies, or were mere 'casual' observations, rather than quantified data. Finally, the characteristics of the environmental enrichments tested differed between studies from vertical or horizontal inserts and

complex burrow systems to additions such as water bottles, flower pots and nesting material.

The search for solutions to modulate aggression between group-housed male mice, other than separating them is encouraged (Council of Europe, 1997). In previous experiments in this respect, we have found that male mice indeed prefer social to individual housing (Van Loo et al., 2001a) and that aggression between males may be decreased by modulating cage cleaning regime and decreasing group size (Van Loo et al., 2000; Van Loo et al., 2001b). Environmental enrichment may also be a tool to reach the objection to decrease aggression between group-housed male mice. In the present experiment, the effect of two types of enrichment, i.e. nesting material or a shelter, were tested for their effect on intermale aggression and on several physiological parameters in groups of male laboratory mice. Both enrichment items have proven to be readily used by mice and we hypothesised that they may decrease aggression between group housed male mice. Kleenex tissues as nesting material (Fig. 1) have been proven to strongly enhance species specific nesting behaviour and were highly preferred by mice compared to nest boxes and other kinds of nesting material (Van de Weerd et al., 1997a, 1998). Nesting material allows mice some level of control over their environment by giving them the opportunity to actively structure their cage. The Utrecht Shelter (Fig. 1) also gives mice a more structured cage. In our experience, this shelter is used for behaviours such as sleeping, eating and defecating. Furthermore, its design is such that mice can use it as a refuge with more than one escape opportunity.

2. Methods

2.1. Animals and husbandry

Forty-five male mice of the BALB/cAnNCRLBr strain were used. The mice were randomly divided in groups of three and housed in wire topped Makrolon II cages (375 cm^2, Tecniplast, Milan, Italy) provided with 50 g sawdust (Lignocel 3/4®, Rettenmaier & Söhne, Ellwangen–Holzmühle, Germany). Tap water and food pellets (RMH-B®, Hope Farms, Woerden, The Netherlands) were provided ad libitum. The animal room had a controlled photoperiod (lights on between 7.00 h and 19.00 h), temperature (23–24 °C), relative humidity ($60 \pm 5\%$), and ventilation (18–20 air changes/h).

At the start of the experiment, the mice were 7 weeks old. The animals were individually marked on the fur with a black waterproof marker (Edding, Germany). The mark was renewed weekly when cages were cleaned. Prior to cage cleaning, food and water were weighed and refreshed, animals were weighed and wounds on tail, back and genitals were counted. Twice-a-week, the sleeping site of the mice was scored between 10.00 and 11.00 h.

When cages were cleaned, five groups of mice were provided with two Kleenex tissues (Fig. 1, Kimberly-Clark Corporation®, EC) torn in strips of 5 cm to ease video analyses, five groups were provided with the Utrecht Shelter (14 cm × 8 cm, 40 mm above the cage floor, mesh size 10 mm × 10 mm; Fig. 1) and five groups served as control with no enrichment. During 12 weeks thereafter, each group alternately received

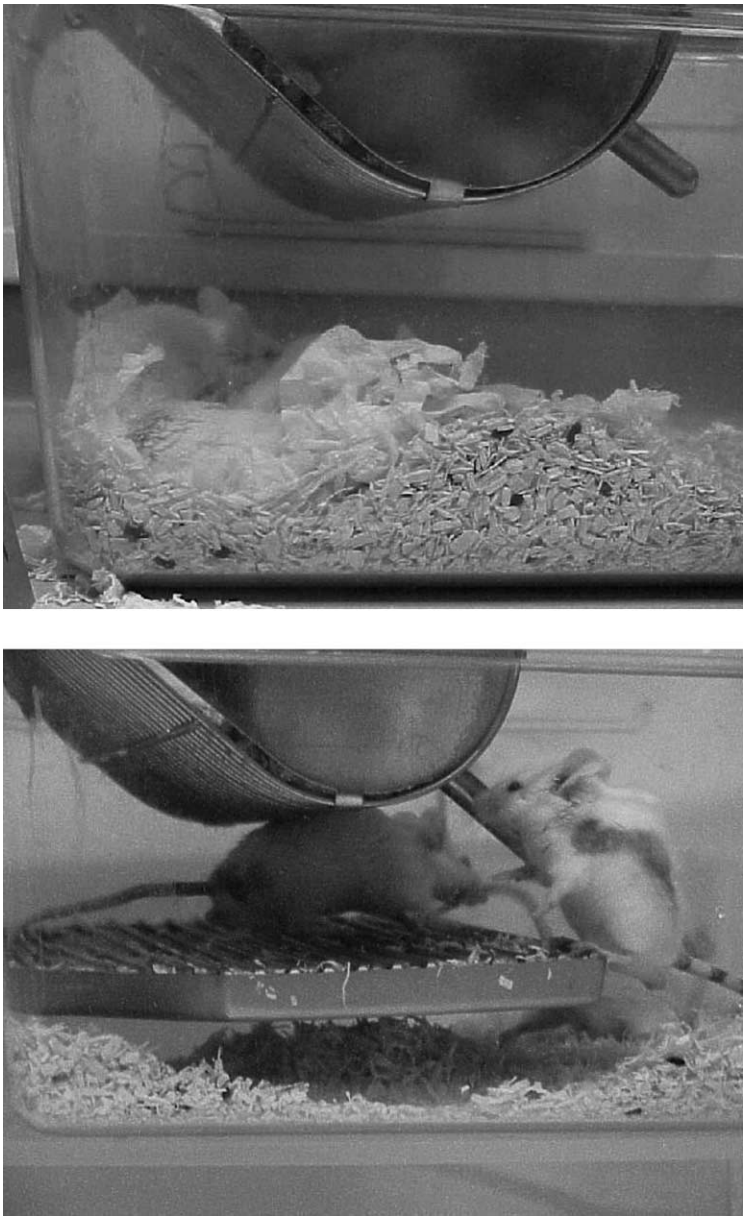

Fig. 1. The two enrichment items tested: Kleenex tissues (top) and The Utrecht Shelter (bottom).

one of the two enrichment devices after cage cleaning, or served as control group according to a previously established randomised block procedure. In this way, each group was subjected four times to each of the three housing conditions in a period of 12 weeks.

2.2. Behavioural data collection

Behavioural data on aggression were collected at two different time periods.

1. *After cage cleaning* (*age of mice*: 7–18 *weeks*). Immediately after transferring the mice to their new environment, their behaviour was recorded on videotape for a period of 30 min. Due to restrictions in the experimental set up, the number of cages cleaned and videotaped simultaneously was limited to four. Videos were taped between 10.00 h and 13.00 h. To minimise influence of time of day on behaviour, order of cages cleaned and recorded was altered weekly according to a previously established randomisation procedure.
2. *After disturbance* (*age of mice*: 16–18 *weeks*). Four days after cage cleaning, all mice were removed from their home cage and isolated for one hour in a plastic bucket covered with a wire top. After this disturbance they were returned to their home cage and their behaviour was recorded on videotape for 30 min. Videos were taped between 10.00 h and 13.00 h. To minimise influence of time of day on behaviour, recording order of cages was altered weekly according to a previously established randomisation procedure.

2.2.1. Behavioural analysis

Latency until first agonistic encounter, frequency and duration of agonistic encounters were scored from videotape. Behaviours interpreted as agonistic were several offensive behaviours such as vigorous sniffing of head, tail or genitals of the opponent, tail rattling, chasing, biting and fighting, and several defensive behaviours such as upright and sideways defensive posture, flee and active defence. Encounters that included biting were marked separately (escalations) as well as encounters that included fighting (fights). The identities of the males involved in an encounter were noted. A male was said to initiate an agonistic encounter when it showed the first agonistic behaviour. A male was said to win an encounter when its opponent showed submissive behaviour terminating the agonistic encounter. Dominant status was assigned to the animal in each group that initiated and won the highest number of encounters. Subordinate status was assigned to the two animals in each group that were attacked most (sub+) or least (sub−).

2.2.2. Urine collection, corticosterone and creatinine analysis

Six days after each cage cleaning, urine samples were collected for corticosterone and creatinine analysis. Between 9.00 and 10.00 h, mice were placed individually in small plastic buckets provided with a disposable plastic dish and a wire top, until the mice urinated, but no longer than 50 min. Urine was collected with a syringe and stored in polypropylene tubes at –20 °C (method described by Dahlborn et al., 1996, and modified by Van Loo et al., 2001b). If mice had not urinated after 45 min. a small layer of ice was put between the bucket and the plastic dish to stimulate urination. Mice that did not urinate at all within 50 min. were subjected to the same procedure the following morning. In this way, the number of missing values could be kept to a minimum.

Corticosterone levels were measured using a solid-phase ^{125}I radioimmunoassay (CAC® Rat Corticosterone TKRC1, Diagnostic Products Corporation, LA). Creatinine

concentrations were determined with the use of a commercial test combination (Creatinine, MA-KIT 10 ROCHE, Roche Diagnostics) on a COBAS-BIO auto-analyser (Hoffmann-La Roche BV, Mijdrecht, The Netherlands).

2.2.3. Organ weights, testosterone levels and tyrosine hydroxylase activity

At the age of 20 weeks, the three animals of each group were euthanised simultaneously by decapitation by three animal technicians between 9.00 and 12.00 h. Decapitation was used to enable blood collection without contamination with anaesthetic compounds. Trunk blood was collected in ice-cooled 1.5 ml reaction vessels containing 50 IU heparin/ml blood. Blood was centrifuged (3000 rpm, 25 min at 20 °C) and serum stored at −20 °C until assayed. Testes, spleen, thymus and seminal vesicles were dissected and weighed (testes and seminal vesicles in pairs). Adrenals were dissected, individually shock-frozen in 5 mM Tris–HCl-buffer (pH 7.2) and stored at −70 °C.

Serum testosterone concentration was measured using a solid phase ^{125}I radioimmunoassay (CAC® Total Testosterone TKTT, Diagnostic Products Corporation, LA). Tyrosine hydroxylase activity (TH) was measured in adrenals using a tyrosine-^{14}C-assay (method described by Witte and Matthaei, 1980).

2.2.4. Statistical analysis

Body weight, food and water intake, organ weights, tyrosine hydroxylase activity and serum testosterone levels as well as most behavioural data were analysed using a multivariate analysis of variance for repeated measures with multiple comparisons. Where necessary, data were logarithmically transformed to better fit the normal distribution. Furthermore, Pearson's correlation (r) was calculated between the total amount of aggression in a group and several physiological parameters (organ weight, TH activity and testosterone level). Number of escalated aggressive encounters, duration of escalations and number of wounds were analysed using a mixed effects analysis of variance with negative binomial error with mouse identity as fixed effect. Urine corticosterone data were logarithmically transformed and analysed using a mixed effects analysis of variance with mouse identity as random effect. For all tests, Bonferroni correction was applied where necessary (indicated by P_B). Number of wounds, corticosterone analyses and analyses of escalations and duration of escalations were carried out with aid of S-plus 2000 Professional Release 2© (1988–1999, MathSoft, Inc.). All other statistical tests were carried out with aid of SPSS for MS Windows Release 9.0 (Chicago Illinois, LA).

3. Results

3.1. Behaviour after cage cleaning

Analyses of behaviour after cage cleaning revealed that frequency and duration of agonistic encounters, latency until the first agonistic encounter, number and duration of escalations significantly differed for different housing conditions (Fig. 2; latency: $P = 0.002$; all other parameters: $P = 0.000$). Contrast results show that nesting material (N) overall reduced agonistic behaviour compared to control housing (C), while the

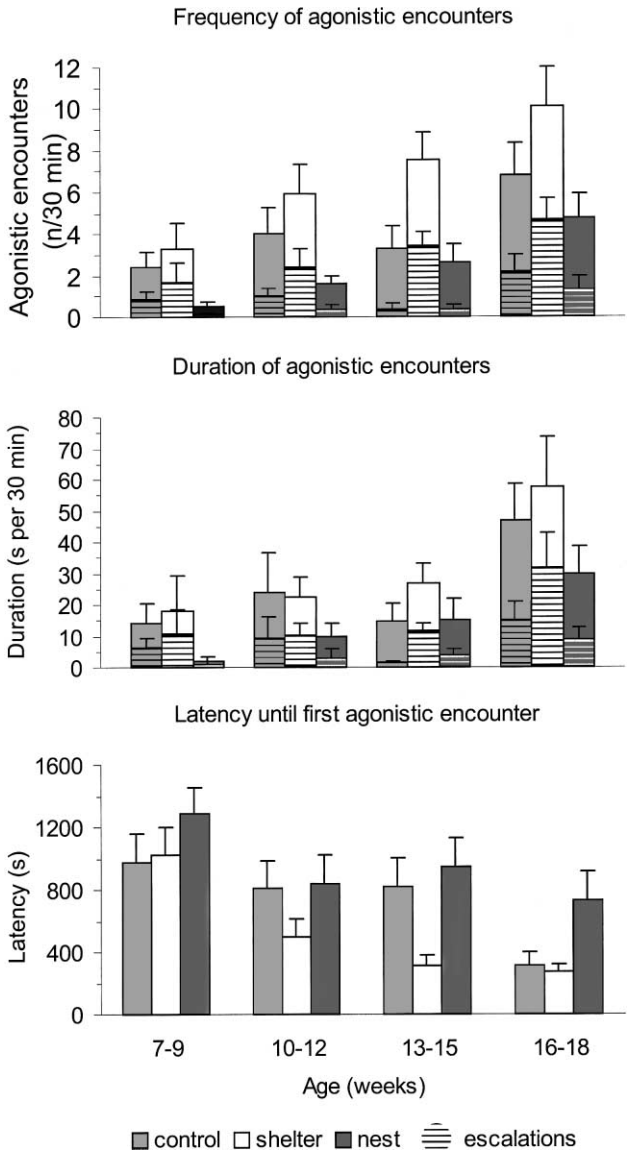

Fig. 2. Frequency (top), duration of agonistic encounters (middle), and latency until first agonistic encounter (bottom) during 30 min after cage cleaning, measured at four different age periods and for three different housing conditions. The striped parts are the amount of encounters that escalated.

Utrecht Shelter housing (S) overall increased agonistic behaviour (latency: $P_{B(S-C)} = 0.096$, $P_{B(N-S)} = 0.015$; frequency: $P_{B(N-C)} = 0.084$, $P_{B(S-C)} = 0.009$, $P_{B(N-S)} = 0.000$; duration: $P_{B(N-C)} = 0.045$, $P_{B(S-C)} = 0.015$, $P_{B(N-S)} = 0.000$; escalations: $P_{B(N-C)} = 0.009$, $P_{B(S-C)} = 0.000$, $P_{B(N-S)} = 0.000$; duration of escalations:

Table 1
Number of fights observed at different ages and in different housing conditions

Age (week)	Housing condition			
	Control	Shelter	Nest	Total
7–9	0	1	0	1
10–12	4	1	0	5
13–15	0	2	0	2
16–18	6	9	6	21
Total	10	13	6	29

$P_{B(N-C)} = 0.000$, $P_{B(S-C)} = 0.079$, $P_{B(N-S)} = 0.000$). Furthermore, there is a clear overall increase in agonistic behaviour with increasing age, while latency until first agonistic encounter decreases with age (latency: $P = 0.010$; all other parameters: $P = 0.000$). Significant interaction effects between age and type of housing were only found in duration of escalations due to a sharp decrease in duration of escalations in the control housing at age 13–15 weeks (Fig. 2; $P = 0.003$). Actual fights were rare and were mainly observed when the mice were 16–18 weeks old (8 out of 15 groups were never observed fighting). No significant differences were found with age or between housing conditions (Table 1).

3.2. Behaviour after disturbance

Analysis of behaviour after disturbance revealed very similar results as behaviour after cage cleaning, however, less pronounced (Table 2). Frequency of agonistic behaviours and number of escalations differed significantly between housing conditions ($P = 0.045$ and $P = 0.004$, respectively), while duration of escalations tended toward the same difference ($P = 0.068$). The largest differences were found between housing with nesting material (N) and shelter (S) and, to a lesser extend, between control housing (C) and shelter (frequency: $P_{B(N-S)} = 0.069$; escalations: $P_{B(N-S)} = 0.006$; $P_{B(C-S)} = 0.10$; duration of escalations: $P_{B(N-S)} = 0.066$). Duration of agonistic encounters, latency until first agonistic encounter and fights did not reveal any significant differences between housing conditions.

Table 2
Behaviour after disturbance (mean ± S.E.M.) for three housing conditions[a]

	Housing		
	Control	Shelter	Nesting material
Frequency of agonistic encounters	12.07 ± 1.76	14.33 ± 1.62 a	9.47 ± 1.42 a
Duration of agonistic encounters	70.13 ± 12.31 a	80.73 ± 11.83 a,b	61.73 ± 13.52 b
Number of escalations	3.93 ± 1.08	7.80 ± 1.09	2.87 ± 0.46
Duration of escalations	29.53 ± 10.19	52.93 ± 9.22 a	25.67 ± 6.68 a
Latency until first agonistic encounter	185.67 ± 88.91	61.93 ± 11.99	148.66 ± 85.81

[a] Similar letters in one row indicate (near) significant differences (a: $P < 0.1$; b: $P < 0.01$).

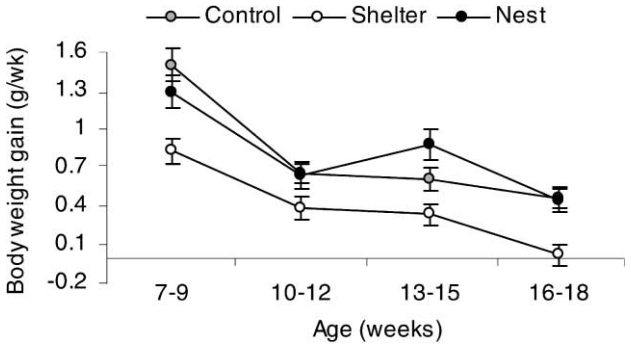

Fig. 3. Body weight gain per week, measured at four different age periods and for three different housing conditions.

3.3. Sleeping site, body weight gain and food and water consumption

When all three mice were resting during scoring of their sleeping site, they were always seen sleeping in close body contact. During control and nest housing, mice usually slept under the food hopper (93 and 89%, respectively), during shelter housing, mice slept (partly) under the shelter in 72% of the cases.

Body weight of the mice increased from 17.1 ± 0.3 g at the start of the experiment to 25.1 ± 0.2 g toward the end of the experiment. Body weight gain decreased significantly with age ($P = 0.000$) and differed significantly for housing condition ($P = 0.000$), but not for status of the individuals. Contrast results showed that during shelter housing, mice gained significantly less weight than during either nest or control housing ($P_{B(N-S)} = 0.000$, $P_{B(C-S)} = 0.000$; Fig. 3).

Both food and water consumption revealed significant effects of housing condition ($P = 0.000$ and $P = 0.010$, respectively; Table 3). Contrast results revealed that when housed with nesting material, mice consumed significantly less food than during control or shelter housing ($P_{B(N-C)} = 0.000$, $P_{B(N-S)} = 0.000$). Furthermore, water consumption was higher during control housing, compared to shelter housing ($P_{B(C-S)} = 0.006$). Both food and water consumption also decreased with increasing age of the mice ($P = 0.000$).

Table 3
Food and water consumption per week for groups of three mice, for four time periods and three housing conditions (mean ± S.E.M.)[a]

Age (week)	Food consumption (g per group per week)			Water consumption (ml per group per week)		
	Control b	Shelter c	Nest b,c	Control d	Shelter d	Nest
7–9	70.79 ± 1.27	71.92 ± 0.84	68.08 ± 1.62	70.89 ± 1.19	68.98 ± 1.13	69.96 ± 0.97
10–12	67.71 ± 1.00	69.27 ± 1.05	64.87 ± 1.27	62.94 ± 0.92	61.14 ± 1.06	61.75 ± 0.92
13–15	65.38 ± 0.94	66.20 ± 0.87	63.38 ± 0.84	63.33 ± 1.66	61.11 ± 1.21	62.50 ± 0.86
16–18	65.53 ± 1.38	65.80 ± 1.31	62.73 ± 1.21	67.37 ± 2.41	63.30 ± 2.15	64.50 ± 2.12

[a] Similar letters indicate significant differences (b, c: $P < 0.001$; d: $P < 0.01$).

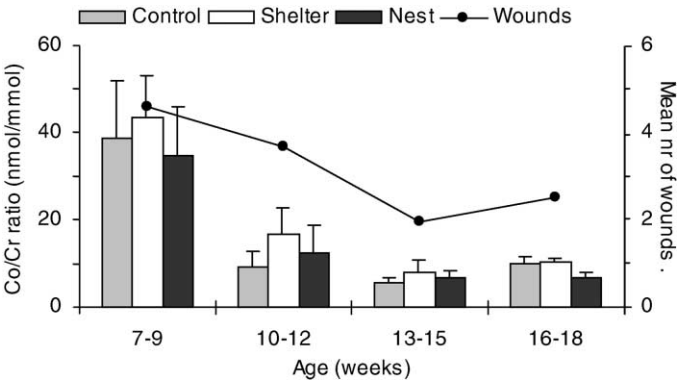

Fig. 4. Urine corticosterone/creatinine ratio's of mice, measured at four different age periods and for three different housing conditions (columns) and mean number of wounds at the same age periods (line).

3.4. Wounds

In concordance with behavioural scores, analyses of number of wounds revealed a clear effect of housing condition ($P = 0.001$). Number of wounds in nest housing was significantly lower than in shelter housing, while control housing was intermediate ($P_{B(N-S)} = 0.028$). Furthermore, number of wounds changed significantly with age (Fig. 4; $P = 0.000$). When the mice were 7–12 weeks old, wound count was quite high, then decreased when the mice were 13–15 weeks old and started to increase again when mice were 16–18 weeks old. There was also a significant age × housing interaction ($P = 0.000$), caused by a sharp increase in wounds during control housing when the mice were 16–18 weeks old. No effects of social status of the mice on number of wounds were observed.

3.5. Organ weights, tyrosine hydroxylase activity and hormone levels

Organ weights, level of testosterone and TH activity were measured post-mortem and could, therefore, not be analysed with housing condition as a possible influential factor. Data are summarised in Table 4. Thymus tended to differ between animals of different status ($P = 0.088$) with the largest difference between the least and most attacked individuals ($P_B = 0.066$). No other differences for organ weights were found. Testosterone levels differed significantly for individuals of different status ($P = 0.012$). Differences were most obvious between least attacked individuals and dominant mice ($P_B = 0.066$). Tyrosine hydroxylase activity did not differ for animals of different status. For none of the above-mentioned physiological parameters, a significant correlation with aggression could be revealed.

Housing condition significantly influenced urine corticosterone/creatinine (Co/Cr) ratios (Fig. 4; $P = 0.003$). Co/Cr ratios in shelter housing were significantly higher than in both control housing and housing with nesting material ($P_{B(C-S)} = 0.024$, $P_{B(N-S)} = 0.005$)

Table 4
Post-mortem variables of mice categorised as dominant (dom), most attacked subordinate (sub+) and least attacked subordinate (sub−)[a]

Parameter	Status		
	Dom	Sub+	Sub−
Organ weights, TH activity and testosterone levels (mean ± S.E.M.)			
Thymus (mg)	38.4 ± 2.1	38.5 ± 1.6 b	43.2 ± 1.7 b
Seminal vesicles (mg)	255.2 ± 8.8	257.5 ± 12.0	243.0 ± 13.1
Spleen (mg)	114.1 ± 9.6	119.6 ± 10.2	104.3 ± 6.5
Testes (mg per pair)	188.1 ± 3.2	191.1 ± 3.7	184.9 ± 3.6
TH activity (nmol/h/adrenal pair)	6.31 ± 0.90	5.41 ± 0.89	5.39 ± 0.52
Testosterone (ng/ml)	21.76 ± 3.87 c	10.57 ± 3.82	6.77 ± 2.27 c

[a] Similar letters within a row indicate a significant difference (b: $P < 0.1$; c: $P < 0.01$).

while the latter two did not differ significantly. Furthermore, Co/Cr ratios changed significantly with age. When the mice were 7–9 weeks old, levels were quite high, decreased when the mice were 10–15 weeks old and started to rise again when mice were 16–18 weeks old ($P = 0.000$).

4. Discussion

4.1. Housing effects

In this experiment, we tested two different types of cage enrichment. Nesting material may satisfy the need of mice for manipulation and nest building. Kleenex tissues have proven to be highly appreciated as nesting material (Van de Weerd et al., 1997a, 1998). A shelter was the second enrichment device tested. The Utrecht Shelter may be used to hide from light and other aversive stimuli such as humans entering the animal room and it enables the mice to eat from a grid floor, which they tend to do more than eating from sawdust when given the choice (Schlingmann et al., 1994). Furthermore, the shelter helps to keep the mice' sleeping area clean of urine and faeces since the animals defecate and urinate mainly on the grid (Blom et al., 1993; Baumans, 2000).

We have found pronounced effects of both enrichments on aggressive behaviour and physiological parameters in male mice. Overall, housing the mice with the shelter increased the amount of intermale aggression and changed a number of stress-related physiological parameters, while housing the mice with nesting material decreased the amount of intermale aggression, indicated by both behavioural scores and number of wounds.

A main difference between the two enrichments tested in this study is the potency for manipulation. The shelter is a rigid, unmanipulative enrichment item, while nesting material can be manipulated and thus, may provide a certain degree of control over the environment. Environmental control is, next to predictability, a very important stress-reducing propensity (Weiss, 1972; Wiepkema and Koolhaas, 1993; Sambrook and Buchanan-Smith, 1997). Nesting material may in this respect satisfy a behavioural need of mice (Poole, 1992; Jensen

and Toates, 1993; Van de Weerd et al., 1997a). It gives mice the opportunity to actively structure their environment and may reduce boredom by building a nest and alleviate social tension and stress by providing a hiding place. Indeed, Chance and Mackintosh (1962) report that the presence of wood wool leads to a considerable decrease in agonistic postures in a confrontation between male mice and Armstrong et al. (1998) found reduced aggression levels in male BALB/c mice that were housed with corn husk nesting material. Although a shelter also provides a structured environment and a hiding place, mice cannot actively change it to meet their satisfaction. Indirect evidence for this can be found in preference tests in which both mice and rats clearly prefer nesting material above rigid structures such as a nest box or a platform (Bradshaw and Poling, 1991; Van de Weerd et al., 1998). In both studies, the nesting material was readily used to build nests. Another fact to be reconsidered is that burrows made by mice in the wild always contain a lot of openings to the surface, providing enough opportunities to flee when the mice are alarmed or threatened (Adams and Boice, 1981). The design of the Utrecht Shelter may have provided insufficient escape routes in this respect, leading to an increase in aggression. Experiments with comparable structures indeed showed increases in aggression (Haemisch and Gärtner, 1994; Haemisch et al., 1994; Bergmann et al., 1994/1995).

The increase in post cleaning and post disturbance aggression during shelter housing in this study was accompanied by an increase in the number of wounds counted, an increase in urinary corticosterone levels, and a decreased weight gain for all mice, irrespective of social status. Both increased (urinary) corticosterone levels and weight reduction are generally used as indicators for chronic or repeated stress situations (Manser, 1992, Brennan et al., 2000). It is noteworthy that many of the escalated encounters that were scored during shelter housing were triggered by tail biting while tails lay on top of the shelter or were hanging down from it. It is possible that the increased visibility of tails during shelter housing accounts for the increase in escalations seen. Bergmann et al. (1994/1995) found an increase in number of wounds counted on mice housed in a labyrinth-like cage with multiple exits. Decreasing the number of exits to two increased the number of wounds even further and the location of wounds differed from on the back of the mice in control housing to mainly on the tail of the mice in the labyrinth-like cage.

During shelter housing, mice drank less. The amount of food consumed when shelter housed, however, tended to be higher rather than lower than during control housing, while weight gain was considerably decreased. Bearing in mind that mice were housed in each condition for only seven consecutive days before entering the next housing condition, these effects on weight gain during shelter housing may be regarded as rather drastic and acute. Similar weight gain reductions have repeatedly been reported for male mice housed in enriched cages with rigid structures such as walls, tubes or shelters (Peters and Festing, 1990; Haemisch and Gärtner, 1994; Bergmann et al., 1994/1995). The difference between food and water intake is also a remarkable phenomenon, as food and water intake are usually in balance (Claassen, 1994). These results, that may indicate a change of the energy balance during shelter housing are in concordance with Leach et al. (2000) who also scored less drinking behaviour in mice housed with a shelter.

During housing with nesting material, on the other hand, mice consumed *less* food than during control housing, while water consumption and weight gain did not differ significantly between these two housing conditions. These results are largely in concordance

with Watson (1993) and Van de Weerd et al. (1997b) who found that mice housed with nesting material consumed less food, while they weighed the same or more than control mice. By using nesting material as insulation to create the preferred microclimate, mice may be able to regulate their body temperature and thus, optimise their energy balance. In the same paper, Van de Weerd et al. (1997b) report no differences in urinary corticosterone ratio's between mice from control and nest housing conditions, a finding also in concordance with findings in the present experiment as well as results of Dahlborn et al. (1996). The increase in urinary corticosterone levels during shelter housing is in concordance with Haemisch and Gärtner (1994) and Haemisch et al. (1994) who found elevated plasma corticosterone levels in mice housed in compartmented cages.

4.2. Age effects

In general, behavioural parameters clearly show an increase in aggression with age. Number of wounds, however, are high at the start of the experiment and, after an initial decrease, rise again towards the end of the experiment. A similar curve can be found for Co/Cr ratio's. It can be argued that in the newly formed groups at the start of the experiment, male mice fought overtly to establish dominance hierarchies, causing wounds and social tension. After dominance hierarchies had been established, males lived in relatively stable environments for a while, causing less arousal. Bronson (1973) too reported an increase in corticosterone levels due to grouping, followed by a decline as groups became stable. As mice matured, however, aggression increased again, possibly because dominant males were challenged regularly, which in turn led to a secondary increase in number of wounds and urinary corticosterone levels. These results are in concordance with results of a previous experiment, in which we found a similar curve in corticosterone levels (Van Loo et al., 2001b) and with Goldsmith et al. (1978) who found increased corticosterone levels due to an increase in fighting.

4.3. Social status effects

For several parameters that showed clear housing effects (i.e. number of wounds, body weight gain and corticosterone levels), no effect of social status could be revealed, indicating that the impact of housing conditions on aggression and stress physiology may be more significant than the impact of hierarchy. In a previous study, no effect of social status on body weight or corticosterone levels was found either (Van Loo et al., 2001b). In this previous study, however, number of wounds clearly differed between dominant and most attacked mice. This apparent discrepancy in results may be explained by the fact that in the previous study, the largest differences between dominants and subordinates were found in groups of five and eight mice, while in groups of three mice (i.e. similar to this study), differences were less pronounced.

We also measured several post-mortem parameters that are known to be influenced by aggression, hierarchy or social stress and we have tried to correlate them to the level of aggression measured in the groups. Contrary to previous results, however (Van Loo et al., 2001a), none of these post-mortem parameters correlated significantly with aggression. Testosterone levels of dominant animals were clearly higher than those of subordinate

animals. This is in concordance with previous results (Van Loo et al., 2000, 2001b) and several other studies (Bishop and Chevins, 1988; Barnard et al., 1994) in which also higher, though not significant, levels of testosterone in dominant mice were found. TH is an enzyme that mediates the transition from tyrosine to dopamine, a precursor for (nor)adrenaline. It provides an estimate of relatively long-term sympathic activity of the adrenal gland (Manser, 1992). We found that TH activity was high in dominant and most attacked subordinate animals and low in least attacked subordinate animals. Although this difference was not significant, its tendency is in concordance with previous results (Van Loo et al., 2001b) and with studies of others (Maengwyn-Davies et al., 1973; Haemisch and Gartner, 1996). These studies seem to indicate that both maintaining dominance (α-males) or being defeated (ω-males) leads to an increase in the sympathic bodily response, while when accepting a subordinate status without ever challenging the α-male this sympathic response is activated less frequent (β-males; Busser et al., 1974).

5. Conclusion

The Utrecht Shelter did not meet our expectations that its structure would reduce aggression in group-housed male mice of the BALB/c strain. Instead, its presence increased aggression and changed several physiological parameters indicative of a stressful situation. It would, therefore, not be advisable to provide this shelter to group housed male laboratory mice at least of the BALB/c strain. Whether the Utrecht Shelter is a suitable enrichment device for female mice or males of other strains remains subject to further study.

Tissues as nesting material have often been proven to be an easy applicable enrichment item that is highly preferred by mice of different strains, both sexes and several different ages. Results of the present study add to these advantages that such nesting material may aid in reducing aggression in group-housed male mice, enabling these social animals to be housed together in laboratory situations where this would otherwise be impossible.

Acknowledgements

The authors would like to thank, K. Brandt, A. van der Sar and R. Timmermans for technical assistance with euthanasia and dissection, and T. Blankensteijn, I. Lemmens and J. Wolfswinkel for assistance with laboratory analyses.

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